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Johnson et al (Arch. Virol. 140:623-634), 1995. Thanks!

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ICP27 immediate early gene, glycoprotein K (gK) and DNA helicase homologues of infectious laryngotracheitis virus (gallid herpesvirus 1) SA-2 strain

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Summary. A 4.8 kilobase segment located at the left-terminal in the unique long (U_L) region of infectious laryngotracheitis virus (ILTV) SA-2 strain contained three open reading frames (ORFs). The first of 421 amino acids (aa) was located at map units 0.065 to 0.07, and its predicted 48 kiloDaltons (kDa) protein product has significant homology to the immediate early regulatory protein ICP27 (UL54) of herpes simplex virus type-1 (HSV-1), to varicella-zoster virus (VZV) ORF4 and to equine herpesvirus 1 (EHV-1) ORF5. The zinc finger conserved in the C-terminal of the proteins from HSV-1, VZV and EHV-1, is poorly conserved in ILTV homologue. The second ORF of 336 aa, located at map units 0.075 to 0.08, has a predicted molecular weight (MW) of 38 kDa with significant homology to glycoprotein K (gK) of HSV-1 (UL53), ORF5 of VZV and ORF6 of EHV-1. ILTV gK has features characteristic of a membrane-bound glycoprotein. The 3′ region of a third ORF was located at map units 0.08 to 0.095. Translation of the sequence revealed significant homology to the 3′-region of the DNA helicase-primase complex protein (UL52) of HSV-1, ORF6 of VZV and ORF 7 of EHV-1. Northern blot analyses were used to characterize the ILTV ICP27, gK and DNA helicase mRNAs. The data revealed that ILTV ICP27 is an immediate early gene that encodes a 1.6 kb mRNA, ILTV gK encodes a late transcript of 1.8 kb, while ILTV DNA helicase encodes a late transcript of 3.7 kb.

Introduction

Infectious laryngotracheitis virus (ILTV) or Gallid herpesvirus 1 is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae* [27, 28]. ILTV can

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cause a severe respiratory disease in chickens primarily affecting the mucous membranes of the larynx and trachea [4]. In the most severe cases, death occurs within 2–3 days after the first signs of illness and can result in mortality rates as high as 70%, although they are usually between 10–40% [4, 11]. The disease is controlled in Australia by the use of a commercial vaccine of a small plaque variant SA-2 [35].

ILTV has previously been shown to have a class D genome of 155 kilobase pairs (kb) characterized by a unique long (U_L) region of 120 kb and a unique short (U_s) region of 17 kb bounded by internal (IR) and terminal repeats (TR) of 9 kb [16, 22]. Random DNA sequencing allowed the identification of 21 ILTV genes by sequence homology to other herpesviruses [9]. Of these genes, 20 had significant sequence homology to genes of varicella-zoster virus (VZV) and 19 to genes of herpes simplex virus (HSV), both alphaherpesviruses. Sequence data of the thymidine kinase (TK) gene and upstream genes provided evidence for an evolutionary relationship between ILTV and other alphaherpesviruses [10].

A complete restriction map for the SA-2 strain is now available [16], and has allowed the positioning of the glycoprotein B gene [18] and other genes such as thymidine kinase, DNA binding protein and the major capsid protein (unpubl. res.). Each of these genes was found in collinear positions with those of the HSV homologues. Similarly, other genes including the immediate early ICP4 homologue [17], protein kinase [20] and a glycoprotein in the gG family [19] were located in collinear positions with the HSV prototype. In this report, we present the sequence data for three genes located between 0.065–0.095 map units at the left hand end of the U_L region, interpret its genetic content and discuss ILTV relatedness to other herpesviruses.

Materials and methods

Growth of ILTV

Primary chicken kidney (CK) cells were obtained from 2 to 4 week old specific pathogen free (SPF) white leghorns and grown in monolayers at 37 °C in minimal Basal Medium Eagle (BME) with Earle's salts, 2 mM glutamine, 100U/ml penicillin, 100 µg/ml streptomycin, 20U/ml polymyxcin, 10 µg/ml neomycin, 2.5 µg/ml fungizone and 10% foetal calf serum. Strain SA-2 of ILTV was supplied by Arthur Websters Pty Ltd. (Castle Hill, Australia) and is the vaccine strain used in Australia. A working stock was obtained by infecting a 10 cm petri dish of CK cells with the initial stock and incubating the cells at 39 °C until they showed >90% cytopathic effect (cpe). Since ILTV is largely a cell-associated virus, the cells were scraped, pelleted, the media removed and the cells resuspended in SPGA (0.2 M sucrose, 0.01 M potassium phosphate, 0.05 M glutamic acid pH 7.0). The titre of the working stock was 5×10^7 PFU/ml. Virus stocks were frozen (liquid nitrogen) and thawed three times prior to infection to release ILTV from cells.

Preparation of ILTV DNA

CK cells in 100 10 cm petri dishes were infected at 1 PFU/cell and incubated at 39 °C until 80–90% cpe. The cell pellet was resuspended in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and extracted twice with NP40 at a final concentration of 1% on ice for 15 min. SDS was

added to a final concentration of 0.5% to the supernatants and was extracted with 1:1 (vol/vol) phenol:chloroform, followed by ethanol precipitation and final resuspension in TE.

Sequencing of DNA

Plasmids used in sequencing were cloned as described previously [16]. Clones containing *Eco*RII (pJJ089) and *Bg*/II fragments pJJ274 and pJJ312 were prepared for double stranded sequencing using the dideoxynucleotide chain termination method [32]. After initial alkali denaturation of the DNA template [12], DNA was labelled using [^{35}S]dATP (Amersham) and the *Taq* polymerase sequencing kit (Promega) and separated on 6% polyacrylamide, 8 M urea buffer. Gels were dried onto glass plates and exposed to Fuji RX film for 1 to 3 days. Oligonucleotides for use in DNA sequencing were made on an Applied Biosystems 392 DNA–RNA Synthesizer, N-butanol precipitated directly from ammonia solution [34] and used without further purification. Both strands were sequenced. Sequences were analyzed using the DNASIS and PROSIS packages (Pharmacia). Alignments were made using CLUSTAL V package [13].

Isolation of mRNA

Monolayers of primary CK cells were prepared in 50 mm petri dishes (1×10^6 cells) and infected with ILTV at a multiplicity of 10 PFU per cell. Cells were treated 2 h prior to infection with cycloheximide (200 μg/ml), then infected for 4 h in the presence of cycloheximide to enrich for immediate early mRNA [14]. To enrich for early mRNA, phosphoacetic acid (PAA) was added (50 μg/ml) to the cell culture medium 6 h post-infection (p.i.) and the mRNA isolated 10 h p.i. No metabolic inhibitors were used to isolate late mRNA with infected cells being harvested 16 h p.i. Poly(A) mRNA from mock- or ILTV-infected CK cells was prepared using the PolyATract mRNA isolation system (Promega).

Northern blot analysis

Poly(A) RNA (0.5 μg) was fractionated on 1% agarose gels containing 2.2 M formaldehyde, 50% formamide as described by Sambrook et al. [33]. After electrophoresis, RNA was transferred onto Hybond−N+nylon filters (Amersham, U.K.) by the method of Southern [36]. RNA molecular weight markers were from Promega. Oligonucleotides were used to PCR the coding regions of the genes, which were purified from agarose gels, labelled with [α-^{32}P] dATP using random hexamers and the Klenow fragment of DNA polymerase (Promega) and hybridized to RNA on filters. DNA-RNA hybridizations were performed at 42 °C for 16 h in 50% formamide, 5 × SSC, 20 mM sodium phosphate (pH 6.5) 1 × Denhardt's solution and 0.1% SDS. Hybridization filters were washed at 42 °C in 0.1 × SSC and 0.1% SDS prior to autoradiography.

Results and discussion

4811 nucleotides of DNA sequence have been lodged with GenBank, accession no L34065. The segment sequenced was located at the left-terminal of ILTV, map units 0.065 to 0.095, and included *Eco*RI fragments I, g′ and a portion of F (Fig. 1).

Gene arrangement and function

Open reading frames (ORFs) were located by searching for ATG initiation codons and candidate polyadenylation sites (AATAAA) near the termini. Three

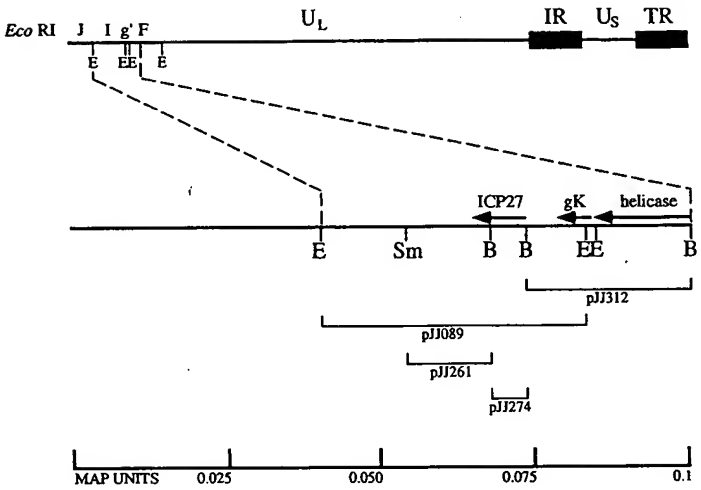

Fig. 1. Map of the infectious laryngotracheitis virus genome. Map unit positions are shown. U_L Unique long region; U_s unique short region; *TR* terminal inverted repeat of the unique short region; *IR* internal inverted repeat of the unique short region. The clones used to sequence the 4811 nt segment reported in this publication are enlarged below the ILTV map. Map locations and direction of ILTV ICP27, gK and DNA helicase homologues are shown

ORFs were found, the direction of transcription was from right to left on the complimentary strand (Fig. 1). The encoded proteins were then compared with translated sequences of HSV-1, VZV, EHV-1, Epstein Barr virus (EBV), channel catfish virus (CCV) and some proteins of Marek's disease virus (MDV), herpesvirus of turkeys (HVT), and pseudorabies virus (PRV), obtained from the GenBank Data library.

ILTV ICP27 homologue

The ORF located at nt 3356 to 4618 was found to be an homologue of HSV-1 UL54 (ICP27; IE63;IE3) [24], VZV ORF4 [7] and EHV-1 UL3 [39] or ORF5 [37] (Fig. 2). The predicted ILTV ICP27 protein is 421 aa and 48 kDa. A potential initiation of transcription motif (TATA) was found 477 bp upstream from the initiation codon and a polyadenlyation signal was located 106 nt downstream of the stop codon. GC rich regions were also found upstream of the start codon. In HSV-1, ICP27 is a 63 kDa alpha phosphoprotein that localizes to the nuclei of infected cells [1]. ICP27 is an essential gene in the regulation of early and late gene expression and is required for virus replication [23, 30]. The hydropathy plot of ILTV ICP27 showed a similar pattern to that of HSV-1 ICP27 [24, 31]. The amino half of the protein is highly hydrophilic (rich in arginine and serine), whilst the carboxyl terminal is relatively hydrophobic. The carboxyl half of HSV-1 ICP27 has been shown to be required for activation and repressor functions in the regulation of early and late gene expression [30]. It has also been shown that a potential zinc finger (metal binding domain) is found in the carboxyl terminal region of HSV-1 ICP27 [24, 38], HSV-1 IE110

[25] and EHV-1 UL3 [39] (Fig. 3). Alignment of the last 100 aa of ILTV ICP27 showed that possibly the first and last cysteine residues of the proposed consensus zinc finger [2,3] are conserved, while the second cysteine and histidine residues have undergone the transitions Cys → Ala and His → Lys. This arrangement in ILTV ICP27 is different from that found in HSV-1, EHV-1 and VZV. However, studies on frame shift mutants showed that the zinc finger motif could be varied and still bind zinc in vitro [38]. Alignment of the ILTV ICP27 protein with ICP27 of HSV-1, ORF4 of VZV and UL3 of EHV-1 showed at the conserved amino acid level homologies of 20, 18 and 18%, respectively, and at the conservative substitution level, 60, 60.5 and 62% respectively. There were also a number of residues conserved across all ICP27 homologues in the carboxyl-terminal. These residues may be important for structure or for activator or repressor functions. Northern blot analysis (Fig. 4A) using clone pJJ274 as a probe showed a 1.6 kb immediate early (IE) transcript of the predicted size and larger transcripts. The 5.4 kb transcript was the result of a previous study on ILTV ICP4. However, we do not know what the other 4.8 kb transcript is, and may be the result of residual protein synthesis in the presence of cycloheximide. Several other transcripts were found early (E) and late (L) in infection (Fig. 4A), and no 1.6 kb transcript was detected. The detection of these transcripts is most likely due to the overlap of sequences in the probe (pJJ261) with 5′ sequence of the next gene downstream from ILTV ICP27. The number of transcripts indicates that several of these genes share 3′ co-terminal mRNAs. As no 5′ or 3′ transcription studies have been done, we do not know where these other transcripts map, or if any products are associated with these transcripts and, therefore, will require a more detailed investigation.

ILTV glycoprotein K (gK) homologue

The ORF located at nt 1537 to 2550 was found to be a homologue of HSV-1 UL53 (gK; cell fusion protein) (DebRoy et al. [8]; McGeoch et al. [24]), VZV ORF5 (Davison and Scott [7]) and EHV-1 ORF6 (Telford et al. [37]) or UL4 (Zhao et al. [39]). No initiation of transcription motif (TATA) was located 5′ to the initiation codon. A polyadenylation signal was located 10 nt 3′ of the stop codon. A GT rich region was also found 24 nt 3′ of the stop codon. These GT regions following polyadenylation signals are associated with RNA cleavage [5]. A second polyadenylation site was located in the 806 bp region between ICP27 and gK. A similar feature was found for the region between HSV-1 UL53 and UL54 (DebRoy et al. [8]; Pogue-Guile and Spear [26]). However, this differed from the region between UL3 and UL4 of EHV-1 (Zhao et al. [39]) and the region between ORF4 and ORF5 of VZV (Davison and Scott [7]) both of which lack a polyadenylation signal and comprise transcripts that shared a 3′ co-terminal (Zhao et al. [39]; Inchauspe et al. [15]). Potential glycosylation sites are located at aa 66 to 68 (NSS) and aa 98 to 100 (NET) (Fig. 5). Trans-membrane domains were located between aa 124 to 145, aa 219 to 235, aa 251 to 273 and aa 306 to 322, and a potential signal sequence was found between aa 1 and 30

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DNA Helicase ->
R  S  D  T  S  I  R  D  S  C  L  L  M  R  S  D  A  P  L  A  D  Q  A  M  L  P  V  I  D  L  P  W  T  E
AGATCTGACACCAGCATACGCGACTCCTGCTTACTAATGCGCAGTGATGCCCCCCTCGCAGATCAGGCCATGTTGCCAGTAATAGACTTGCCTTGGACGG   100

   C  N  E  S  K  P  H  G  E  L  V  R  K  I  K  F  G  Q  S  C  S  G  L  S  Q  M  Y  L  N  R  N  E  V
AGTGCAATGAATCGAAACCGCATGGGGAACTAGTTCGAAAAATAAAATTCGGGCAGTCTTGCAGCGGTCTGTCACAAATGTACCTGAATAGGAATGAAGT   200

   L  N  E  S  L  A  I  Q  S  L  I  L  D  I  D  I  P  L  K  L  D  H  G  P  I  T  M  L  T  L  H  K  A
CCTTAACGAATCTCTAGCAATTCAATCCCTGATACTGGATATCGACATACCCCTCAAGCTAGATCACGGCCCCATAACGATGTTGACCTTACATAAAGCG   300

M  R  A  V  R  V  A  L  I  Q  L  I  A  L  L  F  P  E  T  S  I  S  H  D  T  Y  P  V  Y  F  Y  K  S  H
ATGCGGGCCGTGCGCGTGGCATTGATCCAATTAATAGCCTTATTGTTTCCGGAGACATCAATCAGTCATGACACCTATCCCGTGTACTTTTACAAAAGTC   400

   C  S  S  P  E  S  H  D  A  Q  Y  S  S  V  D  I  D  F  L  E  N  T  C  S  D  D  I  E  A  E  E  N  M
ACTGCTCGTCCCCGGAGAGCCATGACGCACAATATTCCTCAGTGGATATTGATTTTTTGGAGAATACATGTTCTGATGACATAGAAGCAGAAGAGAACAT   500

   Y  V  C  D  S  G  W  V  E  E  M  S  W  C  D  E  H  A  S  E  H  G  A  I  V  S  V  Q  D  L  Q  D  A
GTACGTCTGCGATAGCGGCTGGGTAGAAGAAATGTCATGGTGTGATGAGCATGCGAGTGAACATGGAGCCATAGTCTCTGTTCAAGACTTACAGGATGCG   600

L  N  V  F  R  D  R  A  N  S  T  L  C  K  C  K  E  K  L  G  F  R  V  C  V  P  I  P  S  P  Y  A  L  F
TTAAATGTATTTCGGGATCGTGCCAATTCAACCTTATGCAAATGCAAAGAAAAGCTCGGGTTCAGGGTCTGCGTCCCAATCCCATCCCCATATGCTTTGT   700

   G  L  E  T  V  K  T  I  A  N  V  A  Q  H  V  I  L  L  Q  E  E  F  I  E  C  L  D  D  V  I  R  D  Y
TTGGTCTAGAGACAGTAAAAACTATCGCGAACGTAGCTCAGCACGTAATTTTACTGCAGGAAGAATTCATTGAATGTTTAGACGATGTTATCAGAGACTA   800

   D  F  I  D  T  G  I  Y  S  P  G  R  S  L  R  L  P  F  F  A  K  V  S  E  S  G  F  M  S  G  R  L  L
TGACTTTATTGACACTGGAATCTACTCGCCAGGACGAAGCCTCCGGCTACCTTTCTTTGCCAAGGTTTCTGAATCCGGATTCATGTCTGGGAGACTCCTG   900

P  F  I  I  F  P  P  D  C  A  D  K  V  A  F  A  A  A  H  K  D  P  N  N  F  H  F  H  A  F  R  P  D  N
CCGTTTATAATTTTCCCCCCTGACTGTGCAGATAAAGTAGCTTTCGCGGCAGCGCACAAAGACCCTAATAATTTCCATTTTCATGCTTTCCGTCCAGACA  1000

   P  T  P  N  I  I  V  T  R  I  A  C  P  P  D  V  I  G  G  V  S  K  P  S  S  N  L  P  Q  T  A  V  S
ATCCCACCCCAAATATTATCGTCACTCGAATCGCGTGTCCTCCTGACGTTATAGGGGGAGTTTCAAAACCCTCCAGCAACTTGCCCCAAACGGCTGTGTC  1100

   L  S  L  V  E  A  F  S  R  V  N  L  A  P  C  E  D  R  T  G  A  G  V  D  T  I  D  G  V  F  V  L  T
TCTTAGCCTCGTGGAAGCATTCAGTCGTGTCAATTTAGCACCGTGCGAAGACAGGACAGGAGCCGGGGTGGATACGATCGATGGAGTCTTTGTGCTCACG  1200

T  Y  V  L  P  A  I  T  N  Y  I  K  E  H  F  P  S  L  A  H  E  Y  S  D  I  S  F  G  D  V  R  V  L  K
ACATATGTTCTGCCGGCCATAACAAATTACATAAAAGAGCATTTTCCATCGTTGGCGCACGAATACAGCGACATTAGTTTTGGAGACGTGCGGGTGCTCA  1300

   T  R  I  T  A  S  L  L  R  N  R  R  G  C  G  G  R  A  T  F  T  C  L  K  H  S  H  R  S  A  A  A  Q
AAACGCGCATTACCGCATCACTATTACGAAATCGTCGAGGGTGCGGAGGGCGCGCAACTTTTACCTGCCTTAAACATTCGCATCGAAGTGCCGCTGCCCA  1400

   T  V  I  T  S  V  A  V  A  I  N  S  Q  G  N  P  Y  A  A  F  Q  N  Q  M  L  C  D
GACCGTCATTACTTCTGTAGCGGTTGCAATTAACTCACAGGGCAATCCCTACGCGGCATTTCAGAACCAAATGCTTTGCGACTAAGTGCGGAGGCAACAC  1500

                gK -> M  L  R  P  E  C  L  K  W  A  V  I  L  T  G  T  I  H  I  V  F  L
GCTGCAAACTCAGTTTACAGTGCGTCTGACCAAAGAATGCTGCGACCAGAATGTCTAAAATGGGCAGTAATTTTAACCGGAACGATACATATAGTCTTCT  1600

   V  W  Y  V  C  S  K  I  S  T  D  S  K  D  D  C  L  Y  V  L  A  N  I  K  W  L  L  P  G  Q  Q  E  F
TGGTATGGTATGTATGCTCTAAAATTTCCACTGACTCAAAGGACGACTGCTTGTACGTACTCGCAAATATTAAATGGCTCCTGCCCGGACAGCAAGAATT  1700

   N  P  V  H  Q  P  P  A  T  F  N  S  S  L  V  Y  V  L  T  K  Y  T  Q  R  L  N  N  Y  E  N  H  Q  T
CAATCCTGTTCACCAACCCCCCGCTACATTTAATTCTTCACTAGTTTATGTCCTCACAAAATATACACAGCGTCTAAACAACTACGAGAACCACCAGACG  1800

R  C  V  K  G  A  Y  F  E  N  E  T  V  L  I  S  R  L  I  P  L  A  K  E  Q  Y  S  S  W  K  W  Q  T  V
AGATGTGTTAAAGGAGCTTATTTTGAAAATGAGACTGTACTCATTTCTCGTCTGATCCCACTGGCCAAAGAACAGTATTCGTCATGGAAGTGGCAGACTG  1900

   S  L  H  M  V  F  P  D  Q  S  C  I  S  T  V  I  V  H  M  L  L  A  D  P  C  Q  R  R  M  F  G  S  V
TCTCTTTACATATGGTTTTCCCAGATCAGAGTTGCATTTCCACGGTTATTGTACATATGTTACTTGCTGACCCGTGCCAGAGGCGAATGTTCGGCTCTGT  2000

   C  R  E  N  A  L  R  L  D  A  Y  H  L  N  Y  W  T  A  F  T  S  R  L  I  L  R  V  P  Y  T  K  M  Q
CTGCCGCGAGAACGCATTGCGATTGGATGCATATCATCTAAACTACTGGACAGCGTTTACTTCGAGGCTGATATTACGGGTGCCATACACAAAGATGCAA  2100

R  F  L  R  E  F  E  H  V  R  D  C  K  S  L  N  Y  V  A  D  P  L  G  F  C  I  C  N  P  G  V  L  V  L
CGGTTTTTGAGGGAATTTGAACATGTCCGAGATTGCAAAAGCTTGAACTACGTAGCAGACCCTCTAGGCTTTTGCATCTGTAATCCAGGGGTCTTAGTAC  2200

   K  T  L  E  I  G  L  Y  L  A  S  L  I  M  S  T  M  T  L  R  I  C  Y  D  P  C  A  Y  I  L  H  E  H
TGAAAACACTCGAGATCGGTTTATATTTAGCATCGCTTATTATGTCCACCATGACATTGCGGATTTGCTATGATCCGTGTGCATATATTTTACATGAACA  2300

   V  K  I  S  A  W  V  Y  Y  V  I  V  S  A  V  L  E  L  L  S  L  M  G  Y  T  T  P  A  K  T  K  V  S  A
CGTAAAAATTAGTGCTTGGGTATATGTAATTGTCTCAGCGGTTCTAGAACTCTTATCACTGATGGGTTACACGACTCCGGCAAAGACTAAAGTCTCCGCA  2400

S  K  P  P  S  I  L  T  S  C  L  A  N  I  A  S  S  L  V  L  R  A  L  C  V  A  A  I  A  S  I  V  I  I
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   A  F  K  Y  E  Q  K  I  Q  N  K  L  F  G  P
TTGCATTTAAATACGAACAGAAGATACAAAACAAATTGTTCGGGCCTTGAACGGCAATAAAATGTTAAAACACGTTGTGCGGTGTTGTGTCTGAATTTGG  2600
                                          polyA                  G/T rich region
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TCCATTTGAAGACAGCCCTAGTTCTAACGGCTGAGTATCATTGTTTGAAAGGAGTAACATCTGGCGGTCAGAATGTACAAAGTATACTCGTGGGTGATTT  2700

TAGGGCGTGTTCTACTAAGCGGATGTTTGCGGTTTAGACGGCATGCCGCACTACATTACGGGATTAAGTTACATGTCGGATCCGCAGAAGTTCTCGCAGA  2800

TTATTATGTCTGCGTGAATATTTCGCACCAACTACTGGATCGCAATTGTAGTTTGTGGGTTCCAGTGTATGTGTTATGACGTATACTGGGAGTGGCCCAG  2900
                                                                                    TATA box
ACCATTCAAAAGCCAAGTGGATTAGCTCGCAAACAGCGTGCCAGATTACTGTCAGCTTACCTTTCAGCACCTGCTCCGAGCTTGACACCTGTTCTGAGTC  3000

CGTAGAAGTCTCAACCGTTGTGTATGCGGTTTCATTTTTTTTGACGTGCACGCGGTACATCAACCCACTGACTGCGATGGAGACCGGAGAAATCTGGAAG  3100

AAATAAAGATAGCGTGAGACACGTTCAGAGCATCTTCAATACAAGTACTCATAGCGAAGCGAGATCAGCAACGGCCCGGACAAAAGATCGCCCTGGATGG  3200
polyA
CCGTTCACGCTGAGGTCAGAATCCCGCCACGAAGCACATTGTATTCGCCGCAGATCTCATTCTACAGAAAGTAGCTCCAGATCCCGGTTCATCTGGGCGC  3300
                                                    ICP27 ->  M  A  R  P  R  S  R  Y  E  N  Q  S  G  M  S
GGTACGTCCAAACGAGTCCGTGATTCAAGTGACAGGGGGCGCCGTCGCTGTTCGAATGGCGAGGCCTCGTTCCCGTTACGAGAACCAGTCAGGTATGAGC  3400
                                    G/C rich region
 V  R  S  S  I  R  R  V  S  S  P  R  N  K  F  M  R  G  R  A  H  I  Q  V  R  R  G  I  P  P  R  P  R  R
GTACGGTCTTCCATAAGGCGGGTCAGTAGCCCTCGAAATAAGTTTATGAGAGGCCGTGCGCATATCCAAGTCCGAAGAGGCATCCCCCCTAGACCCAGGC  3500

   R  A  G  T  P  E  K  R  Y  R  A  P  I  F  T  V  S  L  K  H  S  R  R  S  W  E  R  N  R  D  E  L  R
GCCGTGCAGGTACACCAGAAAAGCGATATAGGGCGCCTATCTTTACTGTTTCGTTGAAGCATTCGCGCAGGTCTTGGGAAAGAAATCGGGATGAACTTCG  3600

 R  P  I  W  R  D  F  V  R  C  P  T  S  S  R  T  E  T  K  E  L  R  N  V  T  P  A  Q  Y  F  E  K  A
AAGACCGATTTGGAGAGACTTTGTCCGATGCCCAACGTCATCACGGACCGAAACGAAGGAGTTGCGAAACGTAACACCGGCCCAATATTTTGAAAAGGCC  3700

 A  T  A  F  G  G  L  G  K  C  I  T  E  E  L  R  L  E  N  Q  K  C  L  L  D  M  V  N  R  A  V  D  D  D
GCAACTGCATTTGGCGGTCTCGGAAAGTGCATTACTGAAGAGTTAAGATTAGAAAATCAGAAATGTCTTTTAGACATGGTAAACCGGGCAGTGGATGATG  3800

   D  C  D  D  I  D  R  D  R  G  I  C  F  P  A  F  L  S  S  G  S  S  D  L  A  A  D  A  A  F  T  S  W
ATGATTGTGATGATATCGATCGTGATAGAGGAATCTGCTTTCCAGCATTTTTGTCTTCGGGATCATCTGACCTTGCAGCCGATGCTGCATTCACTTCGTG  3900

 K  Q  F  C  G  R  A  A  S  L  K  G  R  W  T  S  R  P  D  I  A  R  L  A  K  I  S  R  A  V  Y  L  A
GAAGCAGTTTTGTGGGCGCGCAGCTTCACTGAAAGGCCGCTGGACATCGCGTCCGGATATAGCCAGGTTGGCAAAAATTTCACGAGCTGTATATTTGGCG  4000

 N  C  S  F  E  E  L  L  F  A  C  D  E  T  L  V  W  M  L  W  H  Q  F  E  D  E  R  I  Y  P  H  D  P  I
AACTGCTCATTTGAAGAGCTACTTTTTGCATGCGATGAGACACTTGTATGGATGCTTTGGCATCAGTTCGAAGATGAAAGGATTTACCCCCACGATCCTA  4100

   F  S  N  I  Y  C  A  C  Q  S  L  A  M  H  L  G  P  I  L  P  C  Y  L  S  S  I  G  S  Q  L  R  D  T
TCTTCTCTAACATCTACTGCGCATGTCAATCTCTAGCCATGCATCTGGGGCCAATCCTGCCGTGTTATCTCTCTAGCATTGGCAGTCAACTAAGAGATAC  4200

 T  R  S  Q  E  L  S  L  S  S  A  K  C  P  L  T  L  L  L  T  F  F  D  R  F  S  R  I  V  Y  P  R  S
CACTAGATCTCAGGAGCTATCACTGAGTAGCGCAAAATGTCCTTTAACTTTATTACTGACCTTCTTCGACCGGTTCTCAAGAATTGTGTATCCGCGATCA  4300

 E  A  I  V  M  N  H  K  A  I  D  P  A  R  T  L  W  D  M  Y  Y  P  G  T  C  S  K  K  I  P  L  V  L  R
GAGGCCATAGTCATGAATCATAAAGCAATAGACCCGGCCAGAACATTGTGGGACATGTACTATCCTGGGACCTGTTCTAAAAAAATCCCACTCGTTCTGC  4400

   S  T  Q  M  C  A  A  K  R  N  A  E  W  F  V  R  S  S  K  P  Q  Y  T  V  G  K  F  S  L  A  T  C  L
GCAGCACGCAAATGTGTGCCGCAAAGAGGAATGCAGAGTGGTTTGTGCGCAGCTCTAAGCCGCAGTATACAGTAGGGAAATTTTCTCTTGCGACTTGTTT  4500

 T  V  L  Y  T  Y  R  H  M  A  V  L  Y  W  N  W  C  H  P  T  F  L  K  I  A  I  S  V  T  G  Q  Q  M
GACAGTTCTATACACCTATCGACACATGGCGGTATTGTATTGGAATTGGTGCCATCCTACGTTTCTGAAGATTGCCATCTCTGTGACTGGTCAGCAGATG  4600

 A  P  R  A  Q
GCGCCGCGAGCTCAATGATGTAAGCGTTCACTGTTGCAATCCGGCCTTGTAAATACACTGTAGTATTAGCACGGTTGAAAATTTTTAGAATGTTCCCCGA  4700

CTCTCGAAAATTAACGGAGGTATAATAAAGAACACAATACAACGGACACCGACAACGTGGAGCATTTTTATTTAGTCTGCAAATCCTTGGTTGATGAACG  4800
                        polyA
TGGTGCCCTG                                                                                              4811
```

Fig. 2. Nucleotide sequence of the 4811 nt segment containing ILTV ICP27, gK and DNA helicase homologues. *Cis*-acting regulatory sequences such as TATA boxes, polyadenlyation signals (poly A) and GT rich regions are indicated. The deduced amino acid sequence is shown above the nucleotide sequence. Only the 3′ region of the DNA helicase gene is shown

(Fig. 5). The overall conserved amino acid identity with HSV-1 gK, EHV-1 UL4 and VZV ORF5 is 26.7, 25 and 27%, respectively, and at the conservative substitution level, 64.8, 63 and 62%, respectively. Northern blot analysis revealed a faint early transcript of 1.8 kb and a predominant late transcript of 1.8 kb (Fig. 4B). Other faint transcripts of higher molecular weight were also seen and are most likely 3′ co-terminal transcripts, as no polyadenylation signal is found between ILTV gK and the gene 5′.

```
                                                                                a.a.
ILT_ICP27   MAR---------------------------PRSRYENQSGM--------------------  14
HSV_ICP27   MATDIDMLIDLGLDLSDSDLDEDPPEPAESRRDDLESDSSGECSSSDEDMEDPHGEDGPE  60
VZV_ORF4    MASA-SIPTDPDVSTICEDF---MNLLPDEPSDDFALEVTD--WANDEAIGSTPGEDSTT  54
EHV1_orf5   MA--------LSSVSSCEPMEDEMSIMGSDTEDNFTGGDT--CAEATRGLVNKSAFVPTQ  50
            **                              ..  .. ...

ILT_ICP27   ----SVRSSIRRV-----------------------SSPRNKFM---------------  31
HSV_ICP27   PILDAARPAVRPSRPEDPGVPSTQTPRPTERQGPNDPQPA-PHSVWSRLGARRPSCSPEQ 119
VZV_ORF4    S---------RTVYVERTADTAYNPRYSKRRHGR------RESYHHNRPKTLVVVLPDSN  99
EHV1_orf5   TV--GTVSALRNVVGDPPKSVVVSFSASPQRAQPSNPKSERPAFGHGRRNRRRPFRRNNW 108
              . ....* .                                .... ..

ILT_ICP27   ----RGRAHIQVRRGIPPRPRRRAGTPEKRYRAPIFTVSLKHSRR---------------  72
HSV_ICP27   HGGKVARLQPPPTKAQPARGGRRGRRRGRGRGGPGAADGLSDPRRRAPRTNRNPGGPRPG 179
VZV_ORF4    HHGGR---DVETGYARIERGHRRSSRSYNTQSS-------RKHRDRSLSNRRRRPTTPPA 149
EHV1_orf5   KQQQRGWEKPEPENV-PARQSA-GSWPKRS--------SLPVHMRLGQR-----GGDSSS 153
              ... ...  .  ..*..... . .   ..  . .......

ILT_ICP27   -SWERNRDELRRPIWRDFVRCPTSSRTETKELRNVTP---------------AQYFEKAA 116
HSV_ICP27   AGWTDGPGAPHGEAWRGSEQPDPPGGQRTRGVRQAPPPLMTLAIAPPPADPRAPAPERKA 239
VZV_ORF4    MTTGERNDQTHDESYRL--RFSKRDARRERIRKEYDIPV---------------------- 186
EHV1_orf5   ADSGHGGAGP-SDRWRFKTRTQSVA-RVHRNRRRGN------------ANHGSNTPGR-- 197
             .. .  .. .   .*   . ....   ..... ..                .  .  .

ILT_ICP27   TAFGGLGKCITEELRLENQKCLLDMVNRAVDDD--------DCDDIDRDRGICFPAFLSS 168
HSV_ICP27   PAADTIDATTRLVLRSISERAAVDRISESFGRSAQVMHDPFGGQPFPAANS-PWAPVLAG 298
VZV_ORF4    ---DRITGRAIEVVSTAGASVTIDSVRH-LDETIEKLVVRYAT----IQEGDSWAS---- 234
EHV1_orf5   SAGDRLNAAAASSIADVCRRVTSSRIGEMFHGARETLTTPVKNGGFRAENSSPWAPVLGF 257
            .. . .  . . ..  . . .. . ....       ..   . . .... ...

ILT_ICP27   GSSDLAADAAFTSWKQFCGRAASLKGRWTSRPDIARLAKISRAVYLANCSFEELLFACDE 228
HSV_ICP27   QGGPFDAETRRVSWETLVAHGPSLYRTFAGNPRAASTAKAMRDCVLRQENFIEALASADE 358
VZV_ORF4    -GGCFPGIKQNTSWPELMLYGHELYRTFESYKMDSRIARALRERVIRGESLIEALESADE 292
EHV1_orf5   GSDQFNPEARRITWDTLVEHGVNLYKLFEVRSHAAEAARSLRDAVMRGENLLEALASADE 317
            ..... ...  .* .. .... *  ..... ...*.  *  . . .. * * . **

ILT_ICP27   TLVWMLWHQFEDERIYPHDPIFSNIYCACQSLAMHLGPILPCYLSSIGSQLRDTTRSQ-- 286
HSV_ICP27   TLAWCKMCIHHNLPLRPQDPIIGTTAAVLDNLATRLRPFLQCYLKAR----GLCGLDELC 414
VZV_ORF4    LLTWIKMLAAKNLPIYTNNPIVATSKSLLENLKLKLGPFVRCLLLNRDNDLGSRTLPELL 352
EHV1_orf5   TLSWCKMIVTKNLPMRTRDPIISSSVALLDNLRLKLEPFMRCYLSSS----GSPTLAELC 373
            .* *.      .  .  .** .  .  ..*...*.*.. *.*..   ..  .. ..

ILT_ICP27   -ELSLSSAKCPLTLLLTFFDRFSRIVYPRSEAIVMNHKAIDPARTLWDMYYPGTCSKKIP 345
HSV_ICP27   SRRRLADIKDIASFVFVILARLANRVERGVAEIDYATLGVGVGEKM-HFYLPGACMAGLI 473
VZV_ORF4    RQQRFSDITCITTYMFVMIARIANIVVRGSKFVEYDDISCNV-QVL-QEYTPGSCLAGVL 490
EHV1_orf5   DHQRLSDVACVPTFMFVMLARIARAVGSGAETVSRDALGPD-GRVL-ADYVPGACLAGTL 431
             .  ..  ..  . ..  . *....* . ....  .  ... .. . . * **.*

ILT_ICP27   LVLRSTQM-CAAKRNAEWFVRSSKPQYTVGKFSLATCLTVLYTYRHMAVLYWNWCHPTFL 403
HSV_ICP27   EILDTHRQECSS-RVCELTA-SHIVAPPYVHGKYFYC-------------------NSLF- 512
VZV_ORF4    EALITHQRECGR-VECTLSTWAGHLSDARPYGKYFKC-------------------STFN 451
EHV1_orf5   EAIDAHKRRCKA-DTCSLVS-AYTLVPVYLHGKYFYC-------------------NQIF- 470
             .. . .  *.. ....   ..   . ..    *                    ..*

ILT_ICP27   KIAISVTGQQMAPRAQ 429
HSV_ICP27   ---------------- 512
VZV_ORF4    C--------------- 452
EHV1_orf5   ---------------- 470
```

Fig. 3. Alignment of the amino acid sequences of ILTV ICP27, HSV-1 ICP27 (McGeoch et al. [24]), VZV ORF4 (Davison and Scott [7]) and EHV-1 UL3 (Zhao et al. [39]). Numbers at the right indicate the number of amino acids from the N-terminus. Symbols: * conserved amino acids across all for sequences; · conserved or conservative substitutions across two or more sequences

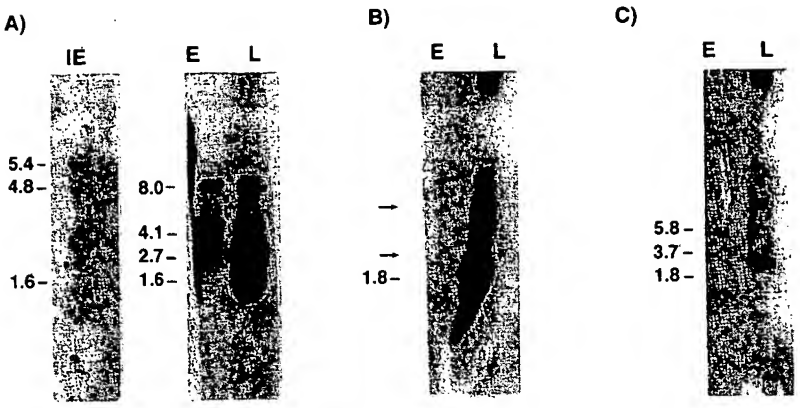

Fig. 4. **A** Northern blot analysis of the ILTV ICP27 transcript. Immediate early (*IE*) mRNA was isolated 4 h post-infection (see Materials and methods). Clone pJJ274 (Fig. 1) was labelled and hybridized as described in Materials and methods. The size of the transcripts are indicated. This filter was previously probed with sequences coding for the ILTV immediate early gene ICP4 (5.4 kb transcript). Clone pJJ261 which contains 3′ sequence of ILTV ICP27 and sequence of putative ORFs downstream was used to probe containing early (*E*) and late (*L*) mRNA. **B** Northern blot analysis of the ILTV gK transcript. Oligonucleotides were used to PCR the open reading frame. This fragment was labelled and hybridized to filters containing early (*E*) and late (*L*) mRNA. The size of the transcript is indicated. **C** Northern blot analysis of the ILTV DNA helicase transcript. Oligonucleotides were used to PCR the 3′ region of open reading frame. This fragment was labelled and hybridized to filters containing early (*E*) and late (*L*) mRNA. The size of the transcripts is shown

DNA helicase homologue

The sequence located at nt 1 to 1485 was found to be homologous to the 3′ region of the HSV-1 UL52 (DNA helicase-primase complex) (McGeogh et al. [24], Crute et al. [6]), VZV ORF 6 (Davison and Scott [7]) and EHV-1 ORF 7 (Telford et al. [37]) (Fig. 2). The DNA helicase induced upon HSV-1 infection consists of three sub-units encoded by UL52, UL5 and UL8. This enzyme is essential for replication and is also associated with DNA primase activity. Northern blot analysis showed several late transcripts of 5.8, 3.7 and 1.8 kb (Fig. 4C). The transcript of 3.7 kb is the predicted mRNA of ILTV helicase, based on the size of the coding region of other alphaherpesvirus helicase homologues (VZV, 3249 nt; EHV-1, 3243 nt). The detection of multiple late transcripts of similar size for both probings of ILTV gK and helicase indicated 3′ co-terminal mRNAs

We have now sequenced a number of genes of ILTV and located their positions on genomic maps of the ILTV SA-2 vaccine strain (Johnson et al. [16]). Based on amino acid sequence homologies it appears that these genes are conserved in relative position between ILTV and other alphaherpesviruses. The arrangement of genes and structure of the left-terminus of ILTV is similar to that found for other herpesviruses. The presence of these highly conserved genes

```
                  signal                                                  a.a.
ILT_gK      ML----RPECLKWAVILTGTIHIVFLVWYVCSKISTDSKDDCLYVLANIKWLLPGQQEFN  56
HSV_UL53    MLAV-RSLQHLSTVVLITA--YGLVLVWY--TVFGASPLHRCIYAVRPT-------GTNN  48
VZV_ORF5    MQALGIKTEHFIIMCLLSG--HAVFTLWY--TA-RVKFEHECVYATTVI-------NGGP  48
EHV1_ORF6   ML-LGGRTAYLSVLGLITA--YAAFTIWY--TL-TAQLHNPCVYATVSI-------DSKD  47
            *.     .. .  ..... . .. .**  .  ... ...*.* .. .      .  .
                 +++                                          +++
ILT_gK      PVHQPPATFNSSLVYVL------TKYTQRLNNYE----NHQTRCVKGAYFENETVLISRL 106
HSV_UL53    DTALVWMKMNQTLLFLGAPTHPPN---GGWRNHAHICYANLIAGRVVPFQVPPDAMNRRI 105
VZV_ORF5    VV---WGSYNNSLIYVTFVNHST--FLDGLSGYDYSCRENLLSGDTMVKTAISTPLHDKI 103
EHV1_ORF6   GIAAKWEVYNSTIVY-AYPENGAKRFSDGLSGFDYVCRENWVNESKLDVLKNMKELHDKV 105
             .     ..*.....        ...   ...    ..   .  .  .  .  .  .  ..
                          124                   145
ILT_gK      -IPLAKEQYSSWKWQTVSLHMVFPDQSCISTVIVHMLLADPCQRRMFGSVCRENAL-RLD 164
HSV_UL53    MNVHEAVNCLETLW-YTRVRLVV-----VGWFLYLAFVALHQRRCMFGVVSPAHKMVAPA 159
VZV_ORF5    RIVLGTRNCHAYFW-CVQLKMIF-----FAWFVYGMYLQFRRIRRMFGPFRSSCELISPT 157
EHV1_ORF6   RIVVGTRNCRAYLW-SVQLQMIT-----GAWLIYIAFLCLRQERRLLGPFRNQNEFLSPT 160
             . ..  .  .. * .. .....     .. ..  . ..   .*...*... .. .
                                                          219
ILT_gK      AYHLNYWTAFTSRLILRVPYTKMQRFLREFEHVRDCKSLNYVADPLGFCICNPGVLVLKT 224
HSV_UL53    TYLLNYAGRIVSSVFLQYPYTKITRLLCELSVQRQNLVQLFETDPVTFLYHRPAIGVIVG 219
VZV_ORF5    SYSLNYVTRVISNILLGYPYTKLARLLCDVSMRRDGMSKVFNADPISFLYMHKGVTLLML 217
EHV1_ORF6   GYTFNYATYTLATTVLKTHYTKFALLLCEASLRRVALSRTFKRDPIGFLCEHSAALALIG 220
            .* .** .   . ..*.  ***. ..* .    *.. .  . .**..*    ... .. .
                  235                 251                 273
ILT_gK      LEIGLYLASLIMSTMTLRICYDPCAYILHEHVKISAWVYVIVSAVLELLSLMGYTTPAKT 284
HSV_UL53    CELMLRFVAVGLIVGTAFISRGACAITYPLFLTITTWCFVSTIGLTELYCILRRGPAPKN 279
VZV_ORF5    LEVIAHISSGCIVLLTLGVAYTPCALLYPTYIRILAWVVVCTLAIVELISYVRPKPTKDN 277
EHV1_ORF6   LEVGTHFVARLLVVGTVTLVHTPCSQIYPIYLKLASWGFVVAVTIVEIVAIIYEKPPKTG 280
             *... ......  .*. . . .*. .    .....*..*.  ...*.....  ......
                                  306             322
ILT_gK      KVSASKPPSIL------TSCLANIASSLVLRALCVAAIASIVIIAFKYEQKIQNKLFG-- 335
HSV_UL53    ADKAAAPGRSKGLSGVCGRCCSIILSGIAVRLCYIAVVAGVVLVALHYEQEIQRRLFDV- 338
VZV_ORF5    HLNHINTG---GIRGICTTCCATVMSGLAIKCFYIVIFAIAVVIFMHYEQRVQVSLFGES 334
EHV1_ORF6   SSANPPTPATHGVKGLCTSCCSTVLANLCGKLVYLLLVIGAVSILLHYEQRIQIGLLGES 340
             .... ...        ..* . . ... .. . .  ....*..  .*** .*  *..

ILT_gK      -----P 336
HSV_UL53    ------ 338
VZV_ORF5    ENSQKH 340
EHV1_ORF6   FSS--- 343
```

Fig. 5. Alignment of the predicted amino acid sequences of ILTV gK, HSV-1 UL53 (DebRoy et al. [8]), ORF5 of VZV (Davison and Scott [7]) and UL4 of EHV-1 (Zhao et al. [39]). Numbers at the right indicate the number of amino acids from the N-terminal. Symbols: * conserved amino acids across all four sequences; · conserved or conservative substitutions across two or more sequences. The potential N-terminal signal sequence and four transmembrane domains for ILTV gK are indicated by lines above the ILTV gK sequence. Potential N-linked glycosylation sites are indicated by +++

in ILTV provides further evidence of the evolution of the alphaherpesviruses from a common progenitor. The levels of conserved identity between the ILTV genes in this study and homologues of other alphaherpesviruses was low, with high levels of conservative substitutions, indicating that considerable divergence has occurred. The reporting of these essential genes provides a framework for future studies on the basic molecular biology of ILTV.

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